U.S. Army Center for Health Promotion and Preventive Medicine

USCHPPM

Wildlife Toxicity Assessment for 1,3,5-Trinitrobenzene (1,3,5-TNB)

NOVEMBER 2001

Prepared by
Health Effects Research Program
Environmental Health Risk Assessment Program

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Department of the Army
U.S. Army Center for Health Promotion and Preventive Medicine

Wildlife Toxicity Assessment for 1,3,5-TNB

CAS No. 99-35-4 **November 2001**

1. INTRODUCTION

1,3,5-Trinitrobenzene (1,3,5-TNB) is one of several compounds that have been released to the environment during the manufacture of explosives and in load, assembly and pack (LAP) activities at U.S. Army ammunition plants (AAPs) and other military installations. The compound has a close structural relationship with the most widely produced military explosive, trinitrotoluene (TNT), of which it is a manufacturing by-product and an environmental degradation product. The importance of 1,3,5-TNB as an environmental contaminant is related to its widespread distribution at and around military sites and to its potential toxicity to wildlife and other ecological receptors. This Wildlife Toxicity Assessment summarizes current knowledge of the likely harmful impacts of 1,3,5-TNB on wildlife, with emphasis on identifying levels at which wildlife species may be adversely effected. Evaluating the toxicity of the compound is intended to contribute to the establishment of toxicity reference values (TRVs) that could serve as protective exposure standards for wildlife ranging in the vicinity of 1,3,5-TNB contaminated sites. The protocol for the performance of this assessment is documented in the U.S. Army Center for Health Promotion and Preventive Medicine Technical Guide 254, the *Standard Practice for Wildlife Toxicity Reference Values* (USACHPPM 2000).

2. TOXICITY PROFILE

2.1 Literature Review

Relevant biomedical, toxicological, and ecological databases were electronically searched May 5, 2000, using Dialog to identify primary reports of studies and reviews on the toxicity of 1,3,5-TNB. Separate searches were carried out linking the compound to either laboratory mammals, birds, reptiles and amphibians (combined), and wild mammals. In general, a two-tiered approach was used in which all citations were first evaluated as titles and “key words in context.” All available abstracts of those articles that were selected in the first tier as possibly relevant to TRV development were then evaluated for relevancy and retention for evaluation in the second tier. For 1,3,5-TNB, 42 articles were marked for retrieval from 259 initial hits, a disparity arising because the initial sweep captured a substantial number of reports that featured the use of trinitrobenzene sulfonic acid to study colitis in laboratory rodents.

These were eliminated in tier 2 of the selection process. Details of the search strategy and the results of the search are documented in Appendix A.

In addition to searching Dialog, a number of U.S. Army reports were identified in the Defense Technical Information Center (DTIC). Secondary references and sources of information on 1,3,5-TNB included an Agency for Toxic Substances and Disease Registry (ATSDR) *Toxicological Profile for 1,3-Dinitrobenzene/1,3,5-Trinitrobenzene* (ATSDR, 1995), the National Library of Medicine's Hazardous Substances Database (HSDB, 2000), the U.S. Environmental Protection Agency's (U.S. EPA) Integrated Risk Information System (IRIS) (U.S. EPA, 2000) and Health Effects Assessment Summary Tables (HEAST) (U.S. EPA, 1997).

2.2 Environmental Fate and Transport

The synthetic compound 1,3,5-TNB is used as a high explosive for commercial mining and military use, as a narrow-range pH indicator and as an agent to vulcanize natural rubber (HSDB, 2000). The compound is a manufacturing by-product of the explosive, TNT, and is released to the environment in discharged wastewater. Additionally, any TNT itself that is present in the waste stream may be degraded to 1,3,5-TNB by photolysis under certain conditions of pH and organic matter content (Talmage et al., 1999). 1,3,5-TNB has been released to the environment during LAP operations performed at AAPs and from open-pit incineration of waste explosives (Simini et al., 1995, Wentsel et al., 1979). Military firing ranges may be contaminated by nitroaromatic compounds from ruptured, but unexploded ordinance. Soil concentrations of up to 67,000 mg/kg have been detected on military reservations (Hovatter et al., 1997).

1,3,5-TNB has an estimated vapor pressure of 3.2×10^{-6} mm Hg at 25°C (Spanggord et al., 1980), a low value implying that partitioning to air is unlikely. 1,3,5-TNB is readily soluble in a variety of organic solvents at ambient temperature, and has a water solubility of 340–385 mg/L at 20–25°C. The compound has been identified in both surface water and groundwater. Furthermore, 1,3,5-TNB has been identified in stream sediments . Hovatter et al. (1997) and Talmage et al. (1999) reported 1,3,5-TNB soil concentration data for certain AAPs, depots and arsenals.

As noted above, TNT will undergo photolysis to produce 1,3,5-TNB in aqueous solution, which is resistant to further photolytic degradation. Nitroaromatic compounds, in general, resist hydrolysis under environmental conditions, with 1,3,5-TNB conforming to this pattern. The various values derived for a K_{oc} (organic carbon/water partition coefficients) for 1,3,5-TNB are estimates, since experimentally determined values are unavailable (Table 1). Talmage et al. (1999) reported K_{oc} values within the range of 76–520 ml/g, indicating a moderate degree of adsorption of 1,3,5-TNB to suspended sediments, and high to moderate soil mobility.

Table 1. Summary of Physical-Chemical Properties of 1,3,5-Trinitrobenzene

CAS No.	99-35-4
Molecular weight	213.11
Color	yellow-white
Physical state	orthorhombic crystals/rhombic plates
Melting point	122.5–125.5 °C
Boiling point	315 °C
Odor	no data
Solubility in water	340–385 mg/L at 20-25 °C: soluble in benzene, methanol, ethanol, ether, and carbon disulfide
Partition coefficients:	
Log K_{ow}	1.18
Log K_{oc}	1.88
Vapor pressure at 25 °C	3.2×10^{-6} mm Hg
Henry's Law constant at 25 °C	3.08×10^{-9} atm.m3/mole
Conversion factors	1 ppm = 8.7 mg/m^3
	1 mg/m^3 = 0.115 ppm

Sources: ATSDR (1995), Talmage et al. (1999), HSDB (2000), Burrows et al. (1989)

1,3,5-TNB is subject to microbial degradation, but the process appears to be limited to the compound's nitrogenous moieties. Thus, aerobic metabolism of 1,3,5-TNB by *Pseudomonas* sp. produces 1,5-dinitroaniline, dinitrobenzene, 5-nitrobenzene, and ammonia. This indicates that 1,3,5-TNB is probably not used as a carbon source by this organism, since no further breakdown was observed (Talmage et al., 1999).

2.3 Summary of Mammalian Toxicity

2.3.1 Mammalian Toxicity - Oral

2.3.1.1 Mammalian Oral Toxicity - Acute

The review by Wentsel et al (1979) cited the Korolev et al. (1977) study on the acute oral toxicity of 1,3,5-TNB in which respective LD_{50} values of 600, 450 and 730 mg/kg were reported for white mice, white rats and guinea pigs (strains unknown). Earlier, Fogelman et al. (1955) determined an LD_{50} of 505 mg/kg for male albino rats (strain unknown) receiving a single gavage dose of 5% weight per volume "TNB" in 0.5% methylcellulose. Moreover, clear-cut indications of cyanosis were described in addition to clinical and neurological effects. Necropsy of study animals that died during the seven-day observation period revealed hemorrhagic lungs, stained kidneys, and a darkening of the blood that was likely indicative of methemoglobin formation. Some of these features were also evident in survivors (number unstated) that were sacrificed at term. More recently, FitzGerald et al. (1991, 1992) conducted a number of acute toxicological tests on military related compounds including 1,3,5-TNB. Five male and female Fischer 344 (F344) rats were exposed to 185, 260 and 335 mg/kg 1,3,5,-TNB in corn oil. Five male and female Swiss mice were similarly exposed to 500, 700 and 900 mg/kg. In addition to results from primary eye and dermal irritation tests, the average acute oral LD_{50} of 1,3,5-TNB for combined sexes was 284 mg/kg in rats and 804 mg/kg in mice (FitzGerald et al., 1991, 1992).

Some acute studies have been conducted with 1,3,5-TNB to investigate endpoints other than lethality. Watanabe et al. (1976) injected nine male Wistar rats intraperitoneally with 100 μmoles/kg 1,3,5-TNB in 2 ml/kg propylene glycol and found formation of methemoglobin in blood samples obtained five hours after dosing. In another study, four male F344 rats per group were given a single oral dose of 1,3,5-TNB (Chandra et al., 1995a). Blood was collected at 5 hours and 24 hours after dosing. The compound was administered in corn oil via oral gavage at doses of 35.5 or 71 mg/Kg. Methemoglobenemia was increased in blood collected 5 hours after administration of 1,3,5-TNB, although other hematological parameters were unchanged. Also, they found significant anemia with reduced red blood cells (RBC), hemoglobin, and hematocrit in rats receiving 1,3,5-TNB for four or ten days. Myers et al. (1999) administered a single oral dose of 50 mg/kg 1,3,5-TNB in corn oil to four male shrews (*Cryptotis parva*) and used gas chromatography/mass spectrometry to demonstrate the formation of hemoglobin adducts in blood samples obtained 24 hours after dosing. *In vitro* incubation of blood with 1,3,5-TNB also resulted in adduct formation; the data suggested a role for cysteine residues in hemoglobin-1,3,5-TNB binding.

Chandra et al. (1995b, 1997) administered a single dose of 1,3,5-TNB in corn oil by gavage to male F344 rats at 35.5 and 71 mg/kg to examine the acute toxicological effects of 1,3,5-TNB on brain and testicular morphology and histopathology. No effects of 1,3,5-TNB on these endpoints were seen in the single dose phase of these studies.

2.3.1.2 Mammalian Oral Toxicity – Subacute

Subacute studies involve repeated dosing of animals and parameters are measured at the end of a14-day study duration. Reddy et al. (1994a, 1996b), using five F344 rats/sex/group, conducted a 14-day oral toxicity study on 1,3,5-TNB to establish suitable dosing levels for longer-term studies. The compound was added to feed to obtain dietary concentrations of 0, 50, 200, 400, 800 and 1200 mg/kg. The respective doses, as calculated by the authors, were 0, 4.52, 16.85, 33.67, 55.76 and 91.93 mg/kg-day in males, and 0, 4.54, 17.5, 34.14, 59.08 and 79.35 mg/kg-day in females. Clinical signs were monitored twice daily, food and water consumption twice weekly, while body weights were recorded at the beginning, at termination, and weekly during the in-life phase of the study. A full suite of hematological and clinical chemistry parameters was assessed in blood samples obtained at necropsy. All tissues and major organs were observed for gross morphological lesions, and the weights of certain key organs were recorded. Samples from numerous internal organs and tissues were fixed and processed for histopathological examination. Sections of sampled tissues from high-dose and control rats were examined under a light microscope, along with sections from all dose groups for potential target organs such as the spleen and kidney.

After treatment the body weights of both sexes of high-dose rats were reduced compared to controls, probably associated with a comparative reduction in food consumption in animals receiving 1,3,5-TNB. Some dose-related changes in organ weight/body weight ratio were evident, including comparative weight increases in brain, spleen and kidneys, and reductions in thymus and testes. The lowest dose at which effects were seen was 16.85 mg/kg-day, a level associated with relative increases in kidney weights in male rats. Though there were no obvious, treatment-related changes in clinical chemistry parameters, most hematological indices were altered compared to controls after 14 days of treatment. For example, a statistically significant and dose-dependent reduction in hematocrit and red blood cell count was observed in all female groups and in males that received at least 55.76 mg/kg-day (RBC decrease) and 33.67 mg/kg-day (hematocrit decrease). Reduced hemoglobin concentrations were observed in female rats that received 59.08 and 79.35 mg/kg-day 1,3,5-TNB. Conversely, methemoglobin increased in a dose dependent manner in both sexes at a dose of approximately 34 mg/kg-day and higher.

There were gross pathological signs in the testes of male rats dosed at 55.76 mg/kg-day and higher, effects that were probably associated with the overall reduction in testicular size. The lesions were marked by moderate to severe seminiferous tubular degeneration, fewer mature spermatids and an apparent reduction in spermatogenic cells. Cell debris and some multinucleate cells were observed in a generally restricted tubular lumen. Another consistent histopathological response to 1,3,5-TNB was the appearance of hyaline droplets in the cortical tubule cells in the kidneys of male rats receiving 16.85 mg/kg-day and above. As described by the authors, many of these large, irregular-shaped droplets were associated with the onset of tubular degeneration (Reddy et al., 1994a, 1996b).

A number of no observed adverse effect levels (NOAELs) and lowest observed adverse effect levels (LOAELs) were evident from the results of this study. For example, a NOAEL of 4.52 mg/kg-day and a LOAEL of 16.85 mg/kg-day corresponded to the histopathological changes in the kidney in male rats (cortical tubular hyaline droplet formation) that were described as occurring in those animals receiving 200 mg 1,3,5-TNB/kg diet and above. However, the most sensitive indices of toxicity were the hematological changes evident in female rats at the lowest dose, which suggests a LOAEL of 4.54 mg/kg-day for these responses (see Table 2).

In another study, four male F344 rats/group received gavage doses of 35.5 and 71 mg/kg 1,3,5-TNB in corn oil for either 4 or 10 days (Chandra et al., 1995a). Blood was collected daily and showed hematological changes similar to those described by Reddy et al. (1994a, 1996b). Using the same protocol, Chandra et al. (1995b) also observed a range of histopathological lesions in the brain of high-dose rats, including gliosis, vacuole formation, malacia, demyelination, edema and hemorrhages, and the formation of petechial hemorrhages in the cerebellar peduncles. No such responses were observed in rats receiving 1,3,5-TNB at 35.5 mg/kg-day. Also, histopathological changes and reductions in testis weights were apparent in male F344 rats, with complete cessation of spermatogenesis in those receiving 71 mg/kg for 10 days (Chandra et al., 1997).

Twelve male and female F344 rats/group and 12 male NCI-Black Reiter (NBR) rats/group were gavaged for 10 days with 1,3,5-TNB in corn oil at 0, 35.5 and 71 mg/kg. Other groups of male F344 rats received 35.5 mg/kg for 20 or 30 days (Kim et al., 1997). The endpoints of the study were the relative incidence of hyaline droplets in the kidney among the groups and an evaluation of the role of the $\alpha 2_{\mu}$-globulin protein in droplet formation. There was dose-dependent hyaline droplet formation in the kidneys of male F344 rats but hyaline droplets were absent from kidney sections of NBR and female F344 rats that had received the same treatments. Immunohistochemical staining with anti-$\alpha 2_{\mu}$-globulin protein anti-serum was apparent in droplet-accumulating regions of the kidneys of affected animals, though mostly confined to the renal proximal convoluted tubular epithelial cells. Hyaline droplets and $\alpha 2_{\mu}$-globulin-positive staining areas were concomitantly absent from reference groups such as treatment-negative controls and female F344 rats and male NBR rats receiving 1,3,5-TNB-treatment negative controls. These observations suggest the possible importance of $\alpha 2_{\mu}$-globulin-related hyaline droplet formation in tubular necrosis and subsequent cellular proliferation of kidneys in male F344 rats dosed with 1,3,5-TNB. They do not, however, address the mechanism by which 1,3,5-TNB induces an increase in kidney weight observed in female F344 and male NBR rats.

Immunohistochemical staining of excised brains was used to evaluate the integrity of the blood-brain barrier in F344 rats challenged with 1,3,5-TNB (Chandra et al., 1999). Five male F344 rats/group were gavaged with 1,3,5-TNB in corn oil at 71 mg/kg for 4, 5, 6, 7, 8 or 10 days with one dose per day. The experiments were carried out to determine whether the vascular bed mediates the pathogenesis of 1,3,5-TNB-induced encephalopathy by comparing the extent of extravasated plasma albumin between treated and control groups. Animals receiving 10 daily doses of 1,3,5-TNB showed an increased intensity and extravascular distribution of immunoreactive albumin in the cerebellum and other parts of the brain, while controls and treated animals granted a recovery period were comparatively unaffected. The authors considered the incidence of vasogenic brain edema to be a critical event in 1,3,5-TNB neurotoxicity.

A fourteen day toxicity evaluation of TNB was conducted in the shrew, *Cryptotis parva*, by feeding 10 animals/group/sex a diet containing 0, 5, 10, 20, and 40 mg/Kg of 1,3,5-TNB (Reddy et al., 2000). The calculated average consumed doses were 0, 10.68, 22.24, 37.79 and 98.27 mg/kg/day for males and 0, 10.75, 21.61, 45.26 and 98.72 mg/kg/day for females. Food and water consumption, body weight and organ weights were measured and hematological and histopathological changes were studied. There were no significant differences in food and water consumption and hematological parameters between control and 1,3,5-TNB fed shrews. The NOAEL for both sexes of shrews was 10.68 mg/kg/day for the oral 14-day study. The LOAEL was 21.6 mg/kg/day for the decreased body and liver weights in males and increased spleen weight in females. (Reddy et al., 2000).

2.3.1.3 Mammalian Oral Toxicity – Subchronic

Reddy et al. (1994b, 1998) described a 90-day toxicological study in 15 F344 rats/sex/group receiving 0, 66.7, 400, or 800 mg 1,3,5-TNB/kg diet, which corresponded to doses, as calculated by the authors, of 0, 3.91, 22.73, and 44.16 mg/kg-day in males and 0, 4.29, 24.7, and 49.28 mg/kg-day in females. A full range of in-life, clinical chemistry/hematological, gross pathological and histopathological evaluations were carried out as previously described for the 14-day study by Reddy et al.(1994a, 1996b).

Critical findings included the formation of kidney lesions characterized by hyaline droplet formation, cortical tubular degeneration and respondent regeneration of tubular cells. These effects were evident in males at all doses. Other responses included methemoglobinemia and erythroid cell hyperplasia in the spleen of high-dose and mid-dose groups (both sexes) and decreased testicular weight with accompanying seminiferous tubular degeneration. Body weight was also significantly less than corresponding controls in males and females at the two highest doses of 1,3,5-TNB. Based on their data the authors considered the most sensitive endpoint to be the kidney lesions evident in male F344 rats and assigned the 3.91 mg/kg-day dose as the subchronic LOAEL for 1,3,5-TNB.

Narayan et al. (1995) investigated the effects of subchronic administration of 1,3,5-TNB on the levels of neurotransmitters and their metabolites in the brain of Sprague-Dawley rats. In a complex protocol, six animals/sex/group received various dietary amounts of 1,3,5-TNB between 0 and 800 mg/kg, equivalent to daily doses of 0, 3, 23 and 51 mg/kg-day in males and 0, 4, 30 and 60 mg/kg-day in females, as calculated by the authors. Exposure duration was 90 days in females and 28 days in males. At necropsy the authors dissected the excised brains into discrete regions, including brain stem, frontal cortex, cerebral cortex, caudate nucleus, septum, hypothalamus, thalamus, hippocampus and cerebellum. Among the neurotransmitters measured were norepinephrine, epinephrine, dopamine, 5-hydroxytryptomine, homovanillic acid, 3,4-dihydroxyphenylacetic acid and 5-hydroxyindoacetic acid.

Compared to controls, levels of some neurotransmitters changed dramatically with treatment. For example, the specific concentration of norepinephrine in the septum increased at least tenfold in both sexes at the lowest dose. Effects such as this suggested a LOAEL of 3 mg/kg-day.

As discussed by Reddy et al. (1997) and on IRIS (U.S. EPA, 2000), Reddy et al. (1995) published an abstract of a meeting presentation in which a 90-day study of 1,3,5-TNB in the White-footed mouse (*Peromyscus leucopus*) was described. Ten animals/sex/group were fed diets of 0, 150, 375 and 750 mg 1,3,5-TNB/kg, with corresponding dose levels calculated to be 0, 23.5, 67.4 and 113.5 mg/kg-day in males and 0, 20, 65 and 108 mg/kg-day in females.

Results from this study included erythroid hyperplasia of the spleen, increased reticulocytes in mid- and high-dose males, and testicular degeneration in the high-dose males, among other effects. Based on the statistically significant change in reticulocyte count in the mid- and high-dose males, 23.5 mg/kg-day is a subchronic NOAEL for 1,3,5-TNB in *Peromyscus*, as suggested in the review by Reddy et al. (1997). This choice differs from a subchronic NOAEL of 67.4 mg/kg-day that was chosen by Talmage et al. (1999) from data on the 1,3,5-TNB-induced testicular effects observed in high-dose animals in the same study. However, both levels of response suggest that the White-footed mouse is less sensitive to the toxic effects of 1,3,5-TNB than F344 or Sprague-Dawley rats.

2.3.1.4 Mammalian Oral Toxicity – Chronic

Reddy et al. (1996a, 2001) used the 14-day and 90-day studies on the toxicity of 1,3,5-TNB in F344 rats to determine suitable dose levels of the compound for a 2-year, chronic toxicity study. Using 60 rats/sex for control groups and 75 rats/sex for test groups, the authors carried out a full range of in-life, clinical chemistry/hematological, gross pathological and histopathological evaluations similar to those described above for their 14-day and 90-day studies (Reddy et al., 1994a, 1994b, 1996b, 1998). However, 10 animals/sex/group were sacrificed after 3, 6, and 12 months for a complete interim histopathological examination of target organs. The levels of 1,3,5-TNB in diet were 0, 5, 60 and 300 mg/kg, which corresponded to calculated dose levels of 0, 0.22, 2.64, and 13.44 in males and 0, 0.23, 2.68, and 13.31 mg/kg-day in females.

Among the key findings, Reddy et al. (1996a, 2001) observed an increased formation of methemoglobin compared to controls in the high-dose groups of either sex. Mid- and high-dose females showed reduced mean corpuscular hemoglobin (MCH), among other hematological fluctuations. While there were no gross necropsy findings related to treatment, histopathological examination of the kidney revealed a myriad of lesions, some probably related to changes in the organ that occur normally in aged F344 rats and some probably related to treatment. For example, necrosis of the cortical tubular cells and formation of tubular cytoplasmic droplets were evident in the mid- and high-dose groups of both sexes. Notwithstanding their appearance in both sexes of F344 rats, these lesions were described by the authors as being "hyaline," large and mostly spheroid in shape and rendered visible with Mallory's Heidenbain protein stain. Immunohistochemical binding to the $\alpha 2_{\mu}$-globulin protein revealed a positive pattern of staining that had a diffuse pattern across the field of view and not necessarily associated with the droplets. The authors concluded that a diagnosis of $\alpha 2_{\mu}$-globulin-related nephropathy would probably be incorrect since there appeared to be no significant increase in tubular cell necrosis. In addition, there were no signs of the granular casts, linear papillary mineralization and tubular hyperplasia that are typical manifestations of this condition.

Increased splenic erythroid cell hyperplasia and pigment deposition were present in mid- and high-dose groups in the interim sacrifices but were restricted to high-dose groups in the 2-year survivors. This suggested that, after 2 years, animals had compensated for the regenerative anemia previously noted in the results from interim sacrifices.

In addition to the effects of 1,3,5-TNB on hematological and histopathological parameters, adults of both sexes at the highest dose were significantly smaller than controls. Body weight at two years was significantly affected by 1,3,5-TNB in both males and females. Decreased body weight was likely the result of decreased food consumption seen in both sexes for all doses of 1,3,5-TNB. However, the effect of the compound on food consumption was significant only for males at the intermediate and high dose levels. It is also possible that smaller body size in 1,3,5-TNB dosed rats may have been the result of alterations in the energy budget of rats resulting from compound-induced toxicity.

The authors considered the methemoglobinemia and splenic erythroid cell hyperplasia with pigment deposition to be the critical effects of 1,3,5-TNB and suggested that a NOAEL of 2.68 mg/kg-day would be appropriate (Reddy et al., 1996a, 2001). This conclusion was set forth in a short review by Reddy et al. (1997) and adopted by the IRIS compilers in their derivation of a oral toxicity reference dose (human health) for 1,3,5-TNB of 3×10^{-2} mg/kg-day (U.S. EPA, 2000).

Reddy et al. (1997) and U.S. EPA (2000) cite a developmental study in Sprague-Dawley rats in which 1,3,5-TNB was administered at 0, 11.25, 22.5, 45 and 90 mg/kg-day by gavage in 1% agar to an unstated number of pregnant females during the major period of organogenesis (gestation days 6–15) (Cooper and Caldwell, 1995). Adverse clinical signs were apparent among the dams, and a number of developmental effects were noted among the pups of dams receiving the highest dose. These included reduced mean fetal weight and crown-rump length, with skeletal variations apparently noted in one animal. These results suggest a developmental NOAEL of 45 mg/kg-day.

2.3.1.5 Mammalian Oral Toxicity – Other

Sprague Dawley rats were used to examine the reproductive impacts of dietary exposure to 1,3,5-TNB (Kinkead et al., 1994a). Ten animals/sex/group received 0, 70, 400 or 800 mg 1,3,5-TNB/kg diet, levels calculated by the authors to result in average doses of 0, 3, 23 and 51 mg/kg-day in males and 0, 4, 30 and 60 mg/kg-day in females (0, 8, 55 and 110 mg/kg-day during lactation). Male rats were dosed for 14 days prior to mating, and then throughout the mating period for a total of 35 days. Female rats were dosed for 14 days prior to mating, and then throughout mating, gestation and lactation, with an additional week post-weaning for a total of 70 days. Pups were maintained on the group-specific treated diet for a total of 7–14 days post-weaning. F_0 male rats were necropsied at termination with sperm count and morphology evaluated in two or three males/group at sacrifice. Testes and epididymides were weighed and examined histopathologically. F_0 female rats were necropsied at termination with pieces of spleen, kidney and liver processed for histopathological evaluation. Blood samples were taken from all groups at termination for methemoglobin analysis. Measures of reproductive performance were noted, including the copulation and fertilization indices and the number of live and dead pups at birth.

A number of the effects of 1,3,5-TNB in this experiment were consistent with those observed in earlier studies described above. For example, there was evidence of enlarged and discolored spleens with hemisiderosis in high- and mid-dose females, testicular atrophy and spermatogenic deficits in high-dose males, and signs of encephalitis in high- and mid-dose females. Neurological signs, for example, head tilt and loss of equilibrium, were observed in lactating dams. Perhaps the most novel findings of the study related to reproductive performance, which, for copulation and fertility indices, were normal, notwithstanding the spermatogenic deficits referred to above. Compared to controls, there were no differences in the F_1 sex ratios and offspring/litter among the groups, although the number of surviving pups after 4 and 21 days were lower than controls in the high-dose group. More than one potential NOAEL can be derived from the results described by Kinkead et al. (1994a). For example, a NOAEL of 4 mg/kg-day may be protective against 1,3,5-TNB-induced encephalitis in female rats (an effect that was evident in mid- and high-dose groups, only), while a NOAEL of 23 mg/kg-day may protect against the testicular atrophy that was evident in high-dose males.

In another reproductive study conducted by the same research group, dietary amounts of 0, 30, 150 and 300 mg 1,3,5-TNB/kg were administered to 18 male and 12 female Sprague-Dawley rats/group for up to 90 days (Kinkead et al., 1994b, 1995). Animals were divided into subgroups to accommodate a number of pre-mating, post-mating and recovery dosing regimen. The doses, as calculated by the authors, were 0, 2, 9 and 19 mg/kg-day for males and 0, 3, 14 and 29 mg/kg-day for females. Subsets of animals were subjected to Opto-Varimex open-field activity evaluation tests, though with no evidence of compound-related depletions in motor skills. At necropsy, samples of blood were taken for clinical chemistry/hematological evaluation. Organ weights were monitored and histopathological evaluations were made of the pituitary, spleen, liver, kidneys, bone marrow and reproductive organs. Sperm counts and motility were measured in comparison to various indices of reproductive performance.

Among the key findings were decreases in sperm motility and degeneration of the seminiferous tubules of the testes in mid- and high-dose males and nephropathy/hyaline droplet formation in males at all dose levels. Methemoglobin was formed in the mid- and high-dose groups for both sexes, although these levels returned to normal in a subset of males allowed to recover after treatment. Splenic hemosiderosis was noted in mid- and high-dose groups of both sexes. By analogy to the findings of Kinkead et al. (1994a), there were no adverse effects of 1,3,5-TNB on mating or fertility, gestation length, sex ratio, or the number of offspring/litter. However, during lactation the body weights of pups borne to high-dose parents were reduced compared to controls. From these data a discriminating index of toxicity appeared to be the changes in sperm morphology and motility to which a NOAEL of 2 mg/kg-day and a LOAEL of 9 mg/kg-day could be assigned. However, 2 mg/kg-day would be a LOAEL for the hyaline droplet-related nephropathy that was evident in male rats at all 1,3,5-TNB dose levels.

2.3.1.6 Studies Relevant to Mammalian TRV Development for Oral Exposures

In the experimental studies described in this review, the overwhelming majority were carried out in laboratory rats, predominantly F344 and Sprague-Dawley strains. Less frequently employed species included the white-footed mouse and the shrew, with no studies identified in birds, reptiles or amphibians. The principal toxicological effects of 1,3,5-TNB are displayed in Table 2 and Figure 1. Responses to 1,3,5-TNB emerging from experimental studies in laboratory animals included (1) nephropathy associated with $\alpha 2_{\mu}$-globulin-associated hyaline droplet formation in male rats (2) atrophy of the testis with associated degeneration of the seminiferous tubules and sperm deficits, (3) structural and functional impairment of the brain, (4) methemoglobin formation, and (5) reduced body weight. These effects are discussed below, in the context of relevancy to TRV derivation.

Nephropathic changes associated with hyaline droplet formation and $\alpha 2_{\mu}$-globulin formation in male rats appear to be a sensitive indicator of 1,3,5-TNB toxicity since no clear-cut NOAEL could be identified for this response in any of the evaluated studies. For example, in Sprague-Dawley rats exposed to the compound for up to 90 days, the lowest dose tested (2.0 mg/kg-day) was identified as a subchronic LOAEL for this response (Kinkead et al., 1994b, 1995). This is a lower value than the chronic NOAEL of 2.68 mg/kg-day emerging from the 2-year study on 1,3,5-TNB with methemoglobinemia specified as the principal effect (Reddy et al., 1996a, 1997, 2001; U.S. EPA, 2000). Unfortunately, data from the 2-year study on 1,3,5-TNB may be unsuitable to shed light on the issue of a chronic NOAEL for $\alpha 2_{\mu}$-globulin-associated hyaline droplet formation and related nephropathy in males, since Reddy et al. (1996a, 2001) described incipient nephropathy and droplet formation in the kidneys of *both* sexes of mid- and high-dose F344 rats. They considered this effect to be inappropriate for a diagnosis of $\alpha 2_{\mu}$-globulin-associated nephropathy in this study, even though gender-specific hyaline droplet formation had been a consistent feature of the toxicological impacts of 1,3,5-TNB in F344 rats (Reddy et al., 1994a,1994b, 1996b, 1998). Since the precise nature and biological significance of the droplets remains uncertain, this endpoint cannot be used for the derivation of a TRV for 1,3,5-TNB.

Impairment of the male reproductive organs with associated decreases in sperm production and motility is a consistent response of experimental animals to nitroaromatic compounds including 1,3,5-TNB. However, similar to the methemoglobinemia, this response appears to be at least partially reversible on cessation of exposure. Moreover, there was no significant effect of 1,3,5-TNB on reproductive performance (Kinkead et al., 1994a,b, 1995). This suggests that these responses may not be critical when deriving quantitative benchmarks for wildlife protection.

Methemoglobinemia can create a functional hypoxic blood condition. Carbon monoxide poisoning also creates a functionally similar condition. Humans with levels of COHb above 10% have reported symptoms of headache while other adverse effects, such as decreased psychomotor performance, were reported when COHb concentrations exceeded 2% (ACGIH 1997). Chronic congenital methemoglobinemia in humans has been found where 10-50% of circulating blood pigment is in the form of methemoglobin with subjects exhibiting no overt signs of toxicity (Smith 1996). In chronic toxicity study, Reddy et al. (1996a, 2001) report differences between control and high dose rats of less than 2%. Given the uncertainty associated with the reported methemoglobin increase in these investigations, increased methemoglobinemia due to 1,3,5-TNB was not considered biologically significant.

Histopathological lesions in various parts of the brain are a consistent feature of responses to 1,3,5-TNB on the part of experimental animals. However, the mechanisms by which such responses are brought about are poorly understood, although the induction of an imbalance in neurotransmitters and their metabolites may play at least a partial role in bringing about these effects (Narayan et al., 1995). No clear links between these histopathological lesions and impaired health or performance were established. Hence, the TRV cannot be based on this response.

Reduced body weight was found in 1,3,5-TNB exposed F344 rats under both subchronic (Reddy et al., 1994b, 1998) and chronic study regimes (Reddy et al., 1996a, 2001). Both studies were high quality with respect to experimental design and execution. A diminution in growth rate may have ecological ramifications by, for example, causing a delay in time to maturity or increasing risk of predation. Reduced somatic growth may not be a direct effect of exposure to 1,3,5-TNB as decreased food consumption was a common response. However, it can be argued that decreased food consumption, as a result of avoidance or direct toxicity, will compromise an organisms function and hence will likely result in altered health or performance. Given the prevalence of reduced body size in 1,3,5-TNB exposed mammals and the likely ecological ramifications of this effect, this endpoint was selected for derivation of the TRV based on data from the chronic study by Reddy et al. (1996a, 2001).

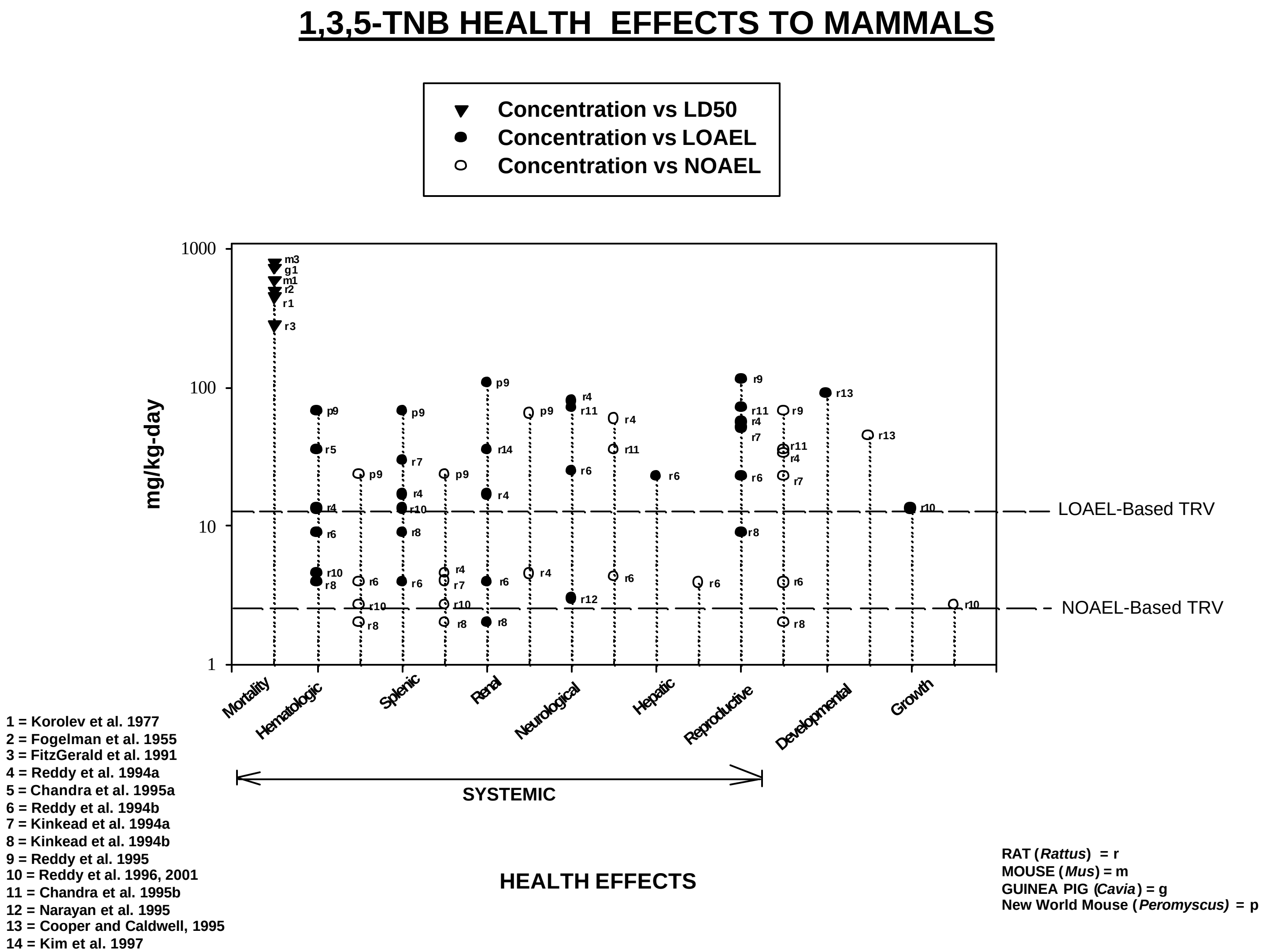
Taken together, the experimental findings summarized in this report present a consistent picture of the overall toxicity of 1,3,5-TNB in experimental animals, with dose levels for toxic effects in the 2–5 mg/kg-day region. Available evidence suggests that the White-footed mouse may be less sensitive to the toxicological effects of 1,3,5-TNB than F344 or Sprague-Dawley rats, although Talmage et al. (1999) used the experimentally derived NOAELs from F344 rats (Reddy et al., 1996a, 2001) and White-footed mice (Reddy et al., 1995) to derive reasonably consistent chronic screening criteria for several wildlife species based on feeding pattern estimates and size comparisons.

Table 2. Summary of Relevant Mammalian Data for TRV Derivation

Study	Test Organism	Test Duration	Test Results		
			NOAEL (mg/kg/d)	LOAEL (mg/kg/d)	Effects Observed at the LOAEL
Reddy et al. (1996a ; 2001)	Rat (Fischer 344)	2-y	2.68	13.31	Methemoglobinemia, spleen erythroid cell hyperplasia. Decreased body weight.
Reddy et al. (1994b; 1998)	Rat (Fischer 344)	90-d	NA	3.91 (m)	Nephropathy/$\alpha 2_{\mu}$-globulin-associated hyaline droplet formation in males at all doses.
			4.29	22.73	Methemoglobinemia, spleen erythroid cell hyperplasia in high-dose and mid-dose groups (both sexes).
Reddy et al. (1994a)	Rat (Fischer 344)	14-d	NA	4.54 (f)	Reduced erythrocyte count and hematocrit in all female groups.
			4.52 (m)	16.85 (m)	Histopathological changes to the kidney in males.
Kinkead et al. (1994b; 1995)	Rat (Sprague-Dawley)	90-d	2.0 (m)	9.0 (m)	Sperm motility/seminiferous tubular degeneration of the testes.
			NA	2.0 (m)	Nephropathy/hyaline droplet formation in males at all doses.
Kim et al. (1997)	Rat (Fischer 344)	10-, 20- and 30-d	NA	35.5 (m)	Nephropathy/$\alpha 2_{\mu}$-globulin-associated hyaline droplet formation in males at all doses.
Narayan et al. (1995)	Rat (Sprague-Dawley)	90-d	NA	3.0	Increase in tissue concentration of various neurotransmitters in several regions of the brain, potentially associated with neurological disorders and histopathological lesions.
Kinkead et al. (1994a)	Rat (Sprague-Dawley)	7-w (m)	23	51	Testicular degeneration and sperm depletion in males.
		10-w (f)	4	23	Encephalitis in females.
Chandra et al. (1995a)	Rat (Fischer 344)	10-d	NA	35.5 (m)	Hematological deficits and methemoglobin formation.
Chandra et al. (1995b)	Rat (Fischer 344)	10-d	35.5 (m)	71 (m)	Histopathological lesions in the brain.
Chandra et al. (1997)	Rat (Fischer 344)	10-d	NA	35.5 (m)	Degenerative changes to the testes.
Cooper and Caldwell (1995)	Rat (Sprague-Dawley)	GDs 6–15	45 (f)	90 (f)	Developmental deficits among the pups.
Reddy et al. (1995)	Mouse (*Peromyscus leucopus)*	90-d	67.4 (m)	113.5 (m)	Testicular degeneration in high-dose males.
			23.5 (m)	67.4 (m)	Erythroid hyperplasia, increase in reticulocyte count in mid- and high-dose males.
Reddy et al. (2000)	Shrew *(Cryptotis parva)*	14-d	10.75 (m)	21.60 (m)	Decrease in liver and body weight.
			10.68 (f)	22.24 (f)	Increase in spleen weight.

NA = not applicable
d = day
w = week
f = female
m = male

Figure 1.


1,3,5-TNB HEALTH EFFECTS TO MAMMALS
Concentration vs LD50
Concentration vs LOAEL
Concentration vs NOAEL
mg/kg-day
1000
100
10
1
LOAEL-Based TRV
NOAEL-Based TRV
Mortality
Hematologic
Splenic
Renal
Neurological
Hepatic
Reproductive
Developmental
Growth
SYSTEMIC
HEALTH EFFECTS
1 = Korolev et al. 1977
2 = Fogelman et al. 1955
3 = FitzGerald et al. 1991
4 = Reddy et al. 1994a
5 = Chandra et al. 1995a
6 = Reddy et al. 1994b
7 = Kinkead et al. 1994a
8 = Kinkead et al. 1994b
9 = Reddy et al. 1995
10 = Reddy et al. 1996, 2001
11 = Chandra et al. 1995b
12 = Narayan et al. 1995
13 = Cooper and Caldwell, 1995
14 = Kim et al. 1997
RAT (Rattus) = r
MOUSE (Mus) = m
GUINEA PIG (Cavia) = g
New World Mouse (Peromyscus) = p

2.3.2 Mammalian Inhalation Toxicity

No inhalation studies conducted using mammals were found.

2.3.3 Mammalian Dermal Toxicity

In vitro percutaneous absorption of 14C-TNB was studied using viable skin from hairless guinea pigs (HGP), F344 rats and human skin in assembled flow-through diffusion cells (Kraeling et al 1998). Results showed that absorption of 1,3,5-TNB in an acetone or water vehicle was rapid, occurring within 24 hrs. The absorption of 1,3,5-TNB in HGP skin was 72% in acetone and 82 % in water. The absorption of 1,3,5-TNB in rat skin was 61% in acetone and 66% in water. However the absorption of 1,3,5-TNB in human skin was 38% in acetone and 75% in water. These results show the absorption of 1,3,5-TNB in acetone was reduced in human skin when compared to rats and HGP. Although this study provided some insight into the percutaneous absorption of 1,3,5-TNB, it did not provide any data on toxicity of this compound when exposure occurs via the dermal route.

2.4 Summary of Avian Toxicology

Toxicological data for the effects of 1,3,5-TNB in avian species was not located. Ecotoxicological research on the effects of this compound in birds is recommended.

2.5 Summary of Amphibian Toxicology

Toxicological data for the effects of 1,3,5-TNB in amphibian species was not located. Ecotoxicological research on the effects of this compound in amphibians is recommended.

2.6 Summary of Reptilian Toxicology

Toxicological data for the effects of 1,3,5-TNB in reptilian species was not located. Ecotoxicological research on the effects of this compound in reptiles is recommended.

3. RECOMMENDED TOXICITY REFERENCE VALUES[1]

3.1 Toxicity Reference Values for Mammals

3.1.1 TRVs for Ingestion Exposures for the Class Mammalia

Decreased body weight, an indication of a lower growth rate, was used to determine the TRV. Growth is an ecologically relevant parameter, which when altered, may affect future fitness. Data on female rats was used because these data were protective of males since females were exposed to slightly lower doses. Moreover, growth as an endpoint is also protective of adverse reproductive effects (Kinkead et al. 1994a,b). Growth data, as indicated by body size, also meet the minimum data requirements of the Standard Practice, Section 2.2 (USACHPPM 2000) and therefore no uncertainty factors are required in the derivation of the TRV. Derivation of the TRV was attempted using the Benchmark dose approach, however, model fit was unacceptable (Appendix B) due primarily to non-homogeneous variance. The NOAEL/LOAEL approach was used and the resulting TRVs are shown in Table 4. This TRV is given a medium confidence rating since there was only one chronic study in F344 rats. Moreover, limited mammalian wildlife toxicity data were available.

Table 4. Selected Ingestion TRVs for the Class Mammalia

TRV	Dose	Confidence
NOAEL-based	2.68 mg/kg/d	Medium
LOAEL-based	13.31 mg/kg-d	Medium

3.1.2 TRVs for Inhalation Exposures for the Class Mammalia

Not Available at this time.

3.1.3 TRVs for Dermal Exposures for the Class Mammalia

Not available at this time

[1] TRVs are for screening purposes only and are not intended to be predictors of effects in field situations. Site specific conditions may justify adjustments of these values based on toxicity information relevant to specific assessment endpoints.

3.2 Toxicity Reference Values for Birds

Not available at this time

3.3 Toxicity Reference Values for Amphibians

Not Available at this time.

3.4 Toxicity Reference Values for Reptiles

Not Available at this time.

4. IMPORTANT RESEARCH NEEDS

Data regarding the toxicity of 1,3,5-TNB are from laboratory rodents, primarily rats, with a few studies on mammalian wildlife species. Hence, the most obvious research need is for studies on the toxicity of 1,3,5-TNB to avian, amphibian and reptile species. Currently, there are no data on these groups of organisms although they are likely to be present at contaminated sites and important components of local habitats. Toxicological testing on these types of organisms would provide data needed to develop TRVs for non-mammalian, ecological receptors.

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APPENDIX A

LITERATURE REVIEW

The following files were searched in Dialog:

File 155 MEDLINE; File 156, TOXLINE, File 5 BIOSIS, File 10 AGRICOLA, File 203 AGRIS, File 399 Chemical Abstracts, File 337 CHEMTOX, File 77 Conference Papers Index, File 35 Dissertation Abstracts, File 40 ENVIRONLINE, File 68 Environmental Bibliography, File 76 Life Sciences Collection, File 41 Pollution Abstracts, File 336 RTECS, File 370 Science, File 143 Wilson Biological & Agricultural Index, File 185 Zoological Record, File 6 NTIS, File 50 CAB, File 144 PASCAL, File 34 SCISEARCH.

The search strategy for **Amphibians & Reptiles**:

- Chemical name, synonyms, CAS numbers
- AND (amphibi? or frog or frogs or salamander? or newt or newts or toad? or reptil? or crocodil? or alligator? or caiman? snake? or lizard? or turtle? or tortoise? or terrapin?)
- RD (reduce duplicates)

The search strategy for **Birds:**

- Chemical name, synonyms, CAS numbers
- And chicken? or duck or duckling? or ducks or mallard? or quail? or (japanese()quail?) or coturnix or (gallus()domesticus) or platyrhyn? or anas or aves or avian or bird? or (song()bird?) or bobwhite? or (water()bird) or (water()fowl)
- RD

The search strategy for **Laboratory Mammals:**

- Chemical name, synonyms, CAS numbers
- AND (rat or rats or mice or mouse or hamster? or (guinea()pig?) or rabbit? or monkey?)
- AND (reproduc? or diet or dietary or systemic or development? or histolog? or growth or neurological or behav? or mortal? or lethal? or surviv? or (drinking()water))
- NOT (human? or culture? or subcutaneous or vitro or gene or inject? or tumo? or inhalation or carcin? or cancer?)/ti,de
- NOT ((meeting()poster) or (meeting()abstract))
- NOT (patient? or cohort? or worker? or child? or infant? or women or men or occupational)
- RD

The search strategy for **Wild Mammals:**

- Chemical name, synonyms, CAS numbers

- And(didelphidae or opossum? or soricidae or shrew? Or talpidae or armadillo? or dasypodidae or ochotonidae or leporidae)or canidae or ursidae or procyonidae or mustelidae or felidae or cat or cats or dog or dogs or bear or bears or weasel? or skunk? or marten or martens or badger? or ferret? or mink? Or aplodontidae or beaver? or sciuridae or geomyidae or heteromyidae or castoridae or equidae or suidae or dicotylidae or cervidae or antilocapridae or bovidae arvicolinae or mycocastoridae or dipodidae or erethizontidae or sigmodon? or (harvest()mice) or (harvest()mouse) or microtus or peromyscus or reithrodontomys or onychomys or vole or voles or lemming?

- AND (reproduc? or diet or dietary or systemic or development? or histolog? or growth or neurological or behav? or mortal? or lethal? or surviv? or (drinking()water))

- RD

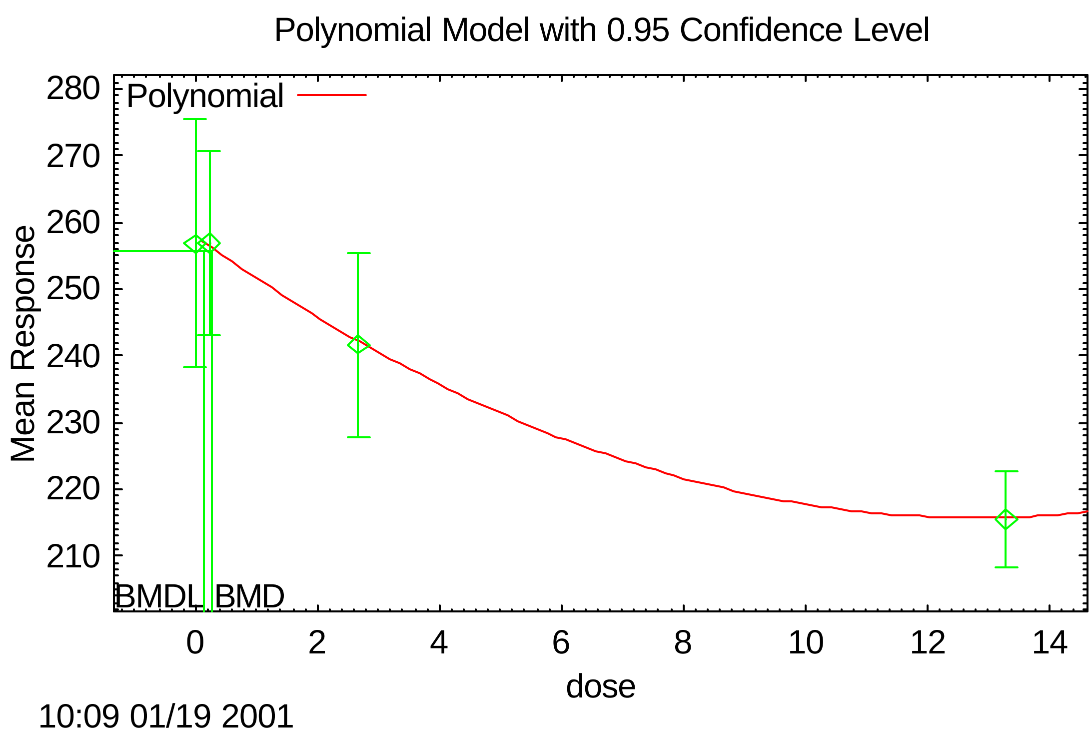
All abstracts from the Dialog search were reviewed and encoded in ProCite. When the search retrieved an appreciable number of hits, *keywords in context* were reviewed to minimize costs before any abstracts were downloaded (Tier 1). However, when only a limited number of studies were identified by the search, the abstracts were downloaded at the time of the search (Tier 2).

As noted in Section 2.1, 259 hits on 1,3,5-TNB were obtained in the initial search, of which 65 were selected for abstract evaluation. Forty-two of these articles and reviews were retrieved for this survey.

APPENDIX B

Benchmark Dose Calculation for Mammals

The data presented below are from Reddy et al. (1996a, 2001) with mean body weight at two years in Fischer 344 rats as the mean response. Data from females were used since the doses used for females were slightly lower than for males and there was a clear dose response. Although the model fit appears adequate, statistically, the model does not fit the data well enough to warrant use of this approach. Test 2 for the constant variance model indicated that variances were not homogenous and suggested using a non-constant variance model. However, the non-constant variance model indicated that the data did not fit the model (Test 3 rejected) and that the variances were inadequately modeled (Test 4 rejected). Both variance models were attempted with various distributions. Hence, it was concluded that the Benchmark Dose approach could not be satisfactorily used. Simulations using the Benchmark Dose Software indicate that another data point (i.e., dose) would likely permit an adequate fit of the data.



The form of the response function is:

Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...

Dependent variable = MEAN
Independent variable = COLUMN1

Signs of the polynomial coefficients are not restricted
The variance is to be modeled as Var(i) = alpha*mean(i)^rho

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha =	380.894
rho =	0
beta_0 =	257.586
beta_1 =	-6.56575
beta_2 =	0.256109

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	176.196	0.0252302
rho	0.121769	24.6244
beta_0	257.624	0.341104
beta_1	-6.58872	2.33176
beta_2	0.25723	30.4537

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	rho	beta_0	beta_1	beta_2
alpha	1	1	0.00026	0.0027	0.0027
rho	1	1	0.00022	0.0026	0.0026
beta_0	0.00026	0.00022	1	0.6	0.52
beta_1	0.0027	0.0026	0.6	1	0.99
beta_2	0.0027	0.0026	0.52	0.99	1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	10	257	26	258	18.6	-0.0427
0.23	10	257	19.3	256	18.6	0.0434
2.68	10	242	19.3	242	18.5	-0.00398
13.31	10	216	10.1	215	18.4	0.00391

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)

Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = alpha*(Mu(i))^rho

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Warning: Likelihood for fitted model larger than the Likelihood for model A3.

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-136.743	5	283.486
A2	-132.792	8	281.585
A3	-477.62	6	967.241
fitted	-136.682	5	283.364
R	-149.344	2	302.687

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	33.1024	6	<.0001
Test 2	7.90151	3	0.04809
Test 3	689.656	2	<.0001
Test 4	-681.877	1	<.0001

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .05. A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is less than .05. You may want to consider a
different variance model

The p-value for Test 4 is less than .05. You may want to try a different
model

Benchmark Dose Computation
Specified effect = 0.1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.950000

BMD = 0.285666

BMDL = 0.151617

BMDL computation failed for one or more point on the BMDL curve.
The BMDL curve will not be plotted